

Outline of talk

- Covert inflammation
- Biomarkers of nutritional status influenced by inflammation
 - Transient effects
 - Chronic effects
- Correcting data for influence of inflammation

What do we mean by inflammation?

- The changes that occur in our bodies in response to an infection.
- Infection or tissue damage stimulates an acute phase response that is designed to protect us against the invading organism or the microbes that might invade following the damage
- The changes are essentially protective

Detecting sub-clinical inflammation

- Infection increases cytokines IL-1, IL-6 and TNF and these stimulate the liver to synthesise
- A number of acute phase proteins that appear and disappear at different times relative to the period of infection
- Acute phase proteins are relatively stable & useful biomarkers of disease/stress

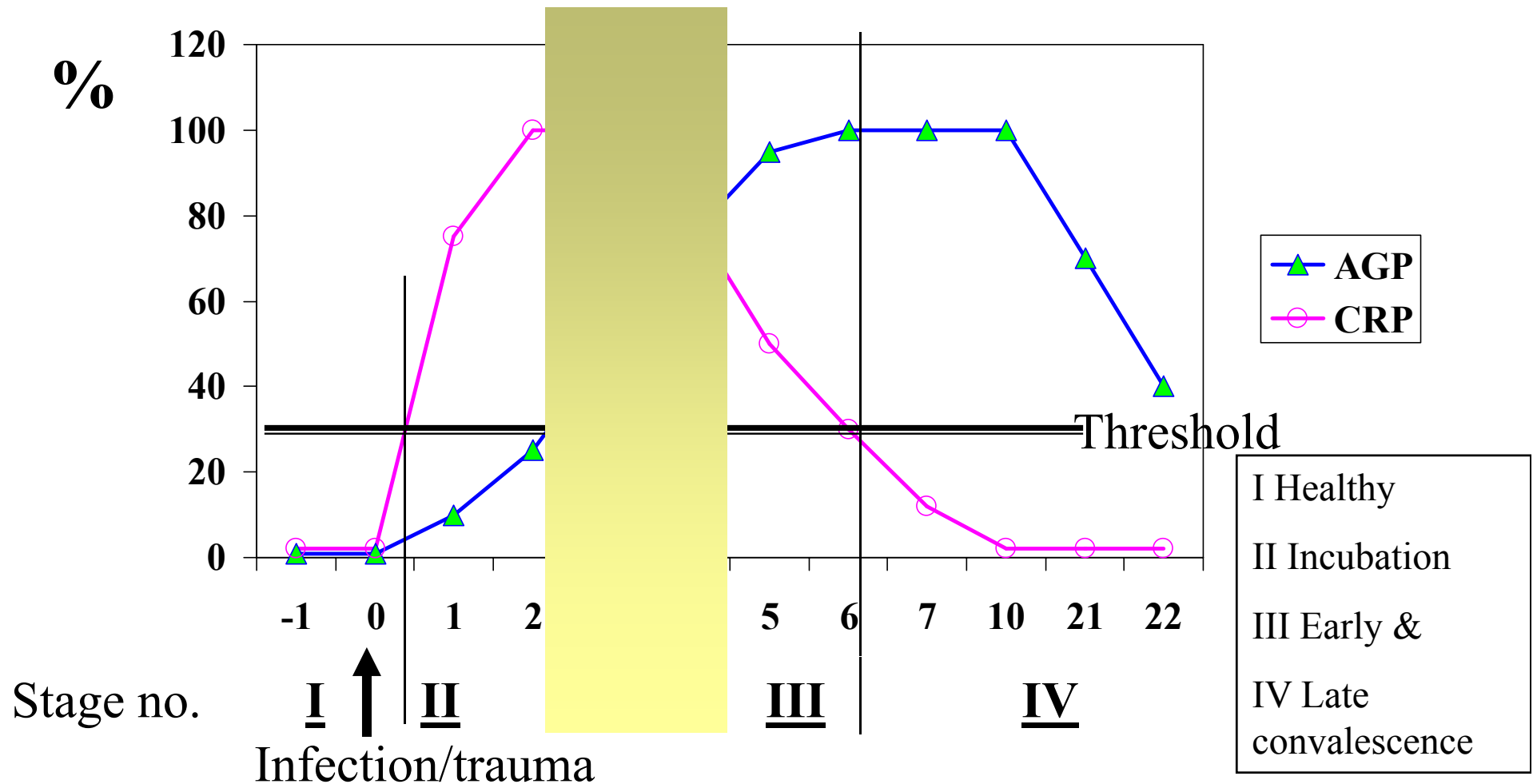
Useful acute phase proteins

Plasma C-reactive protein (CRP) increases rapidly within 5 hours of infection. Is maximal between 24 to 48 hours and falls rapidly with disappearance of symptoms.

A2

Plasma α -1 acid glycoprotein (AGP) concentration is slower to rise. Unusual to detect before 48 hours and is not maximal until 4-5 days following infection

Time course of acute phase proteins following onset of infection



Inflammation

- Precedes, accompanies and follows clinical effects of disease & physical trauma
- **Covert** – Inflammation is present in apparently healthy people
- Point about inflammation of greatest relevance to nutritionists is
- Nutritional status is assessed in the 'apparently-healthy' people

Blood biomarkers influenced by inflammation

Biomarker

- Blood concs of **retinol**, vitamin C, carotenes, pyridoxal phosphate, **iron**, **zinc**, selenium
- Blood concs of **ferritin**, transferrin receptors & erythrocyte (zinc) proto-porphyrin

Effect of inflammation

- Depressed
- Increased

Consequence of inflammation on nutritional status

Depressed Biomarker

- Blood concs of **retinol**, vitamin C, carotenes, pyridoxal phosphate, **iron, zinc**, selenium

Raised Biomarker

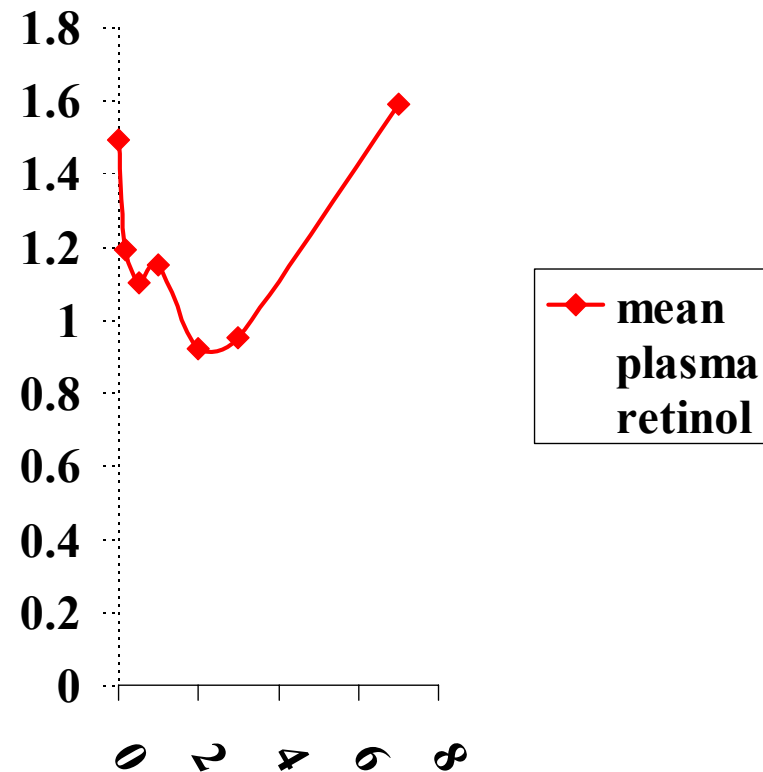
- Blood concs of **ferritin**, transferrin receptors & erythrocyte proto-porphyrin

Effect of inflammation

- Elevate the prevalence of deficiency
- Decrease the prevalence of deficiency

Temporal effects of inflammation

When the infection/inflammation is acute the effects on biomarkers of nutritional status are transient



Louw et al 1993

Temporal effects of inflammation

Where there is chronic infection or frequent infections the effects on nutritional status may persist

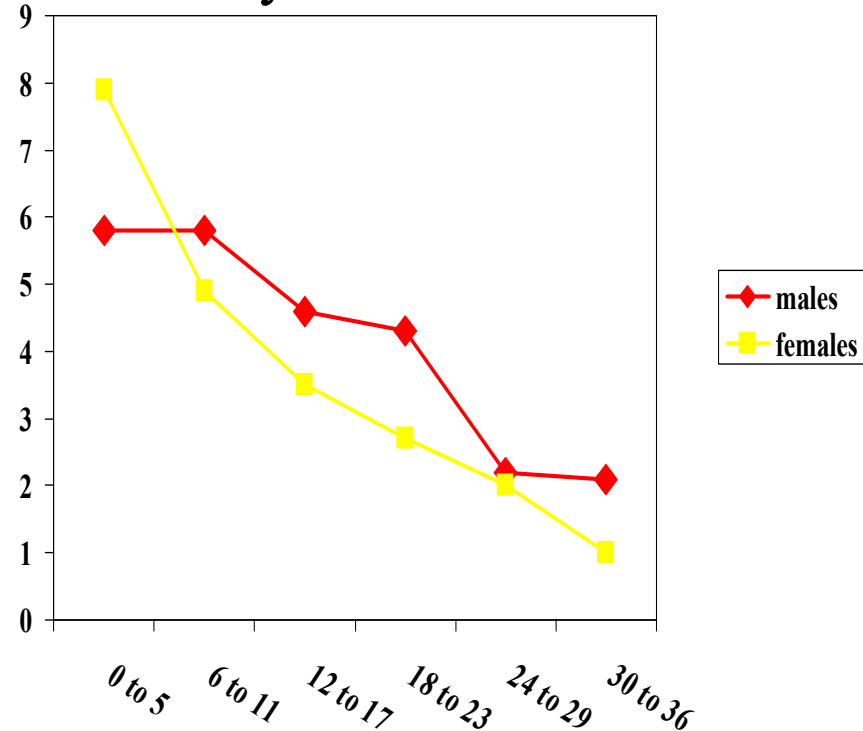
Anaemia of infection or inflammation is the obvious example e.g. cancer or rheumatoid arthritis

But

? Children in developing countries

Prevalence of disease in children

Acute episodes
diarrhoea /year



Sepulveda et al
1988

Months

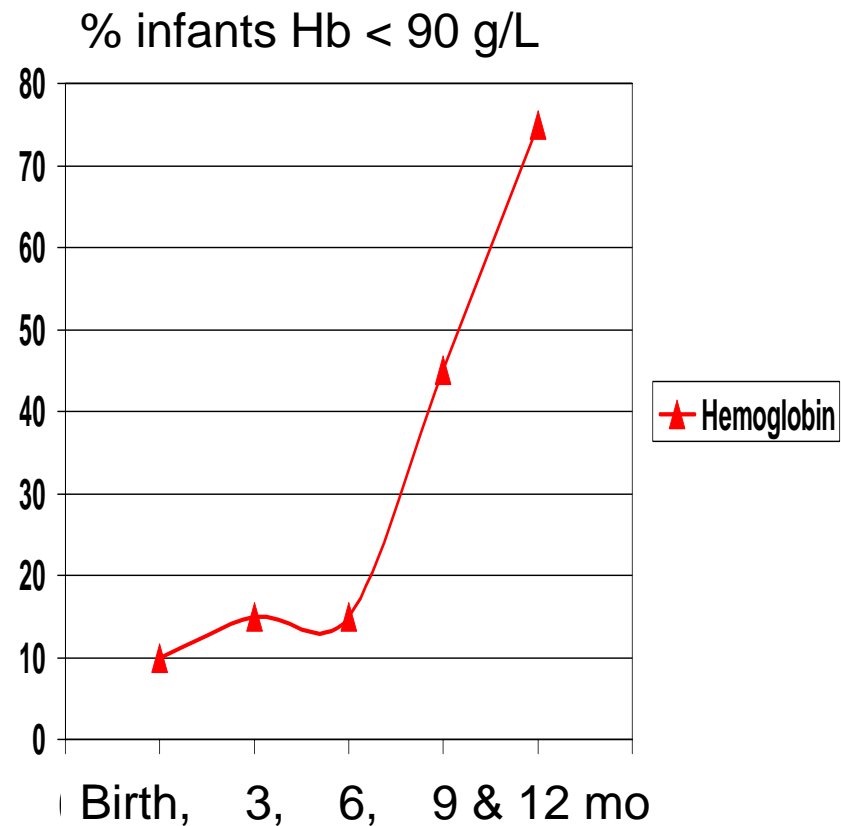
- In The Gambia, Rowland et al (1977) reported infants have 6-8 acute GI episodes/y & spend
- 13% of time ill with GI and
- 10% of time with upper respiratory tract infections
- In PNG Shankar et al (1999) reported children bitten by malaria-positive mosquitoes 4 x/wk

Anaemia is also very common in Gambian infants

Is the anaemia due to iron deficiency or infection?

There was very little anaemia up to 6 months but at 9 mo 45% of infants had mild anaemia increasing to 75% at 12 mo

All Hb measurements on apparently-healthy infants



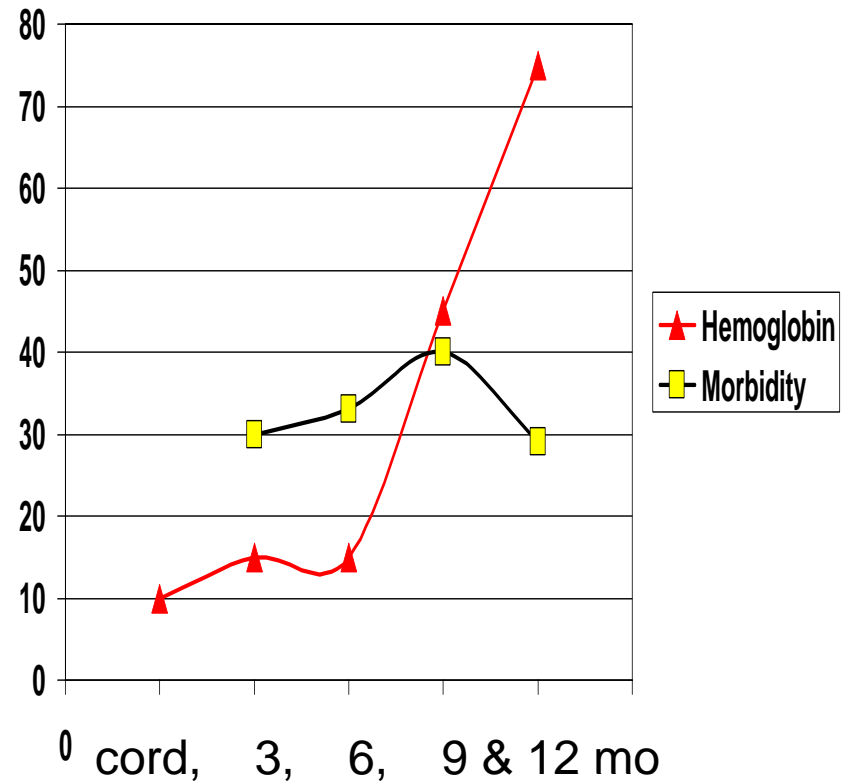
Darboe et al Lancet 2007;368:2088

Morbidity and anaemia in Gambian infants

Morbidity was measured at home twice weekly

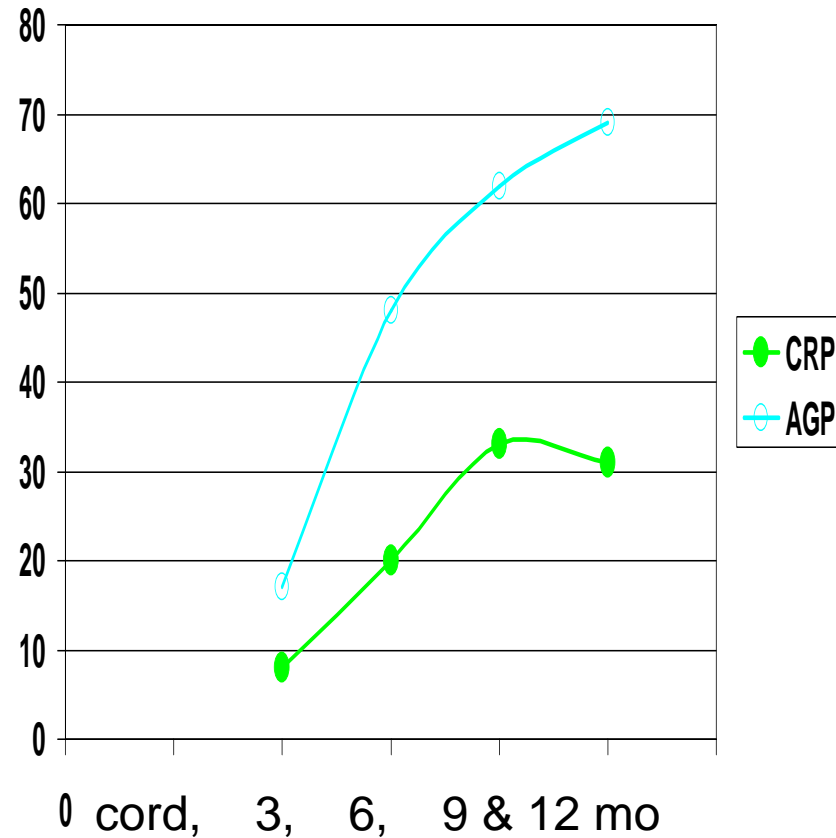
Figures show no. of days sick in 3 months

The prevalence of morbidity hardly changed over 12 mo



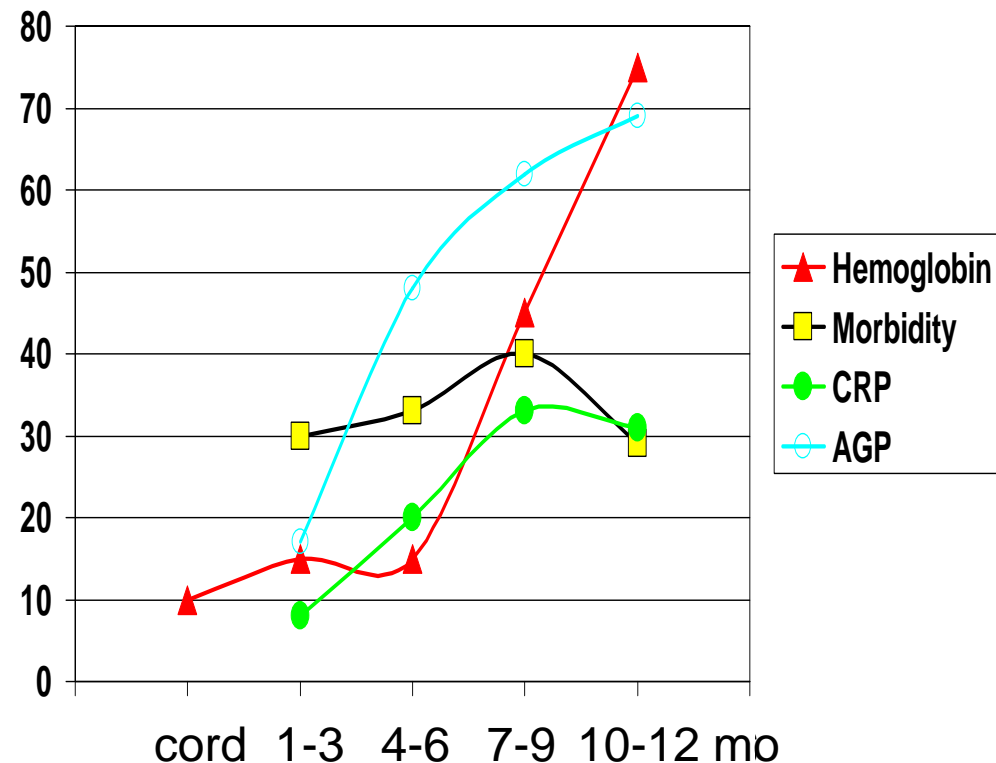
Prevalence of sub-clinical inflammation in apparently-healthy Gambian infants

- Prevalence of raised AGP and CRP was ~10% at 3 mo
- Prevalence continued to increase at 6 and 9 mo
- High AGP values still rising at 12 mo



Total morbidity, Hb, CRP & AGP in Gambian infants

- Morbidity: sick days in 3 mo
- Haemoglobin: % infants < 90 g/L
- CRP: % > 5 mg/L
- AGP: % > 1 g/L
- Acute inflammation CRP corr more closely with morbidity
- AGP is cumulative

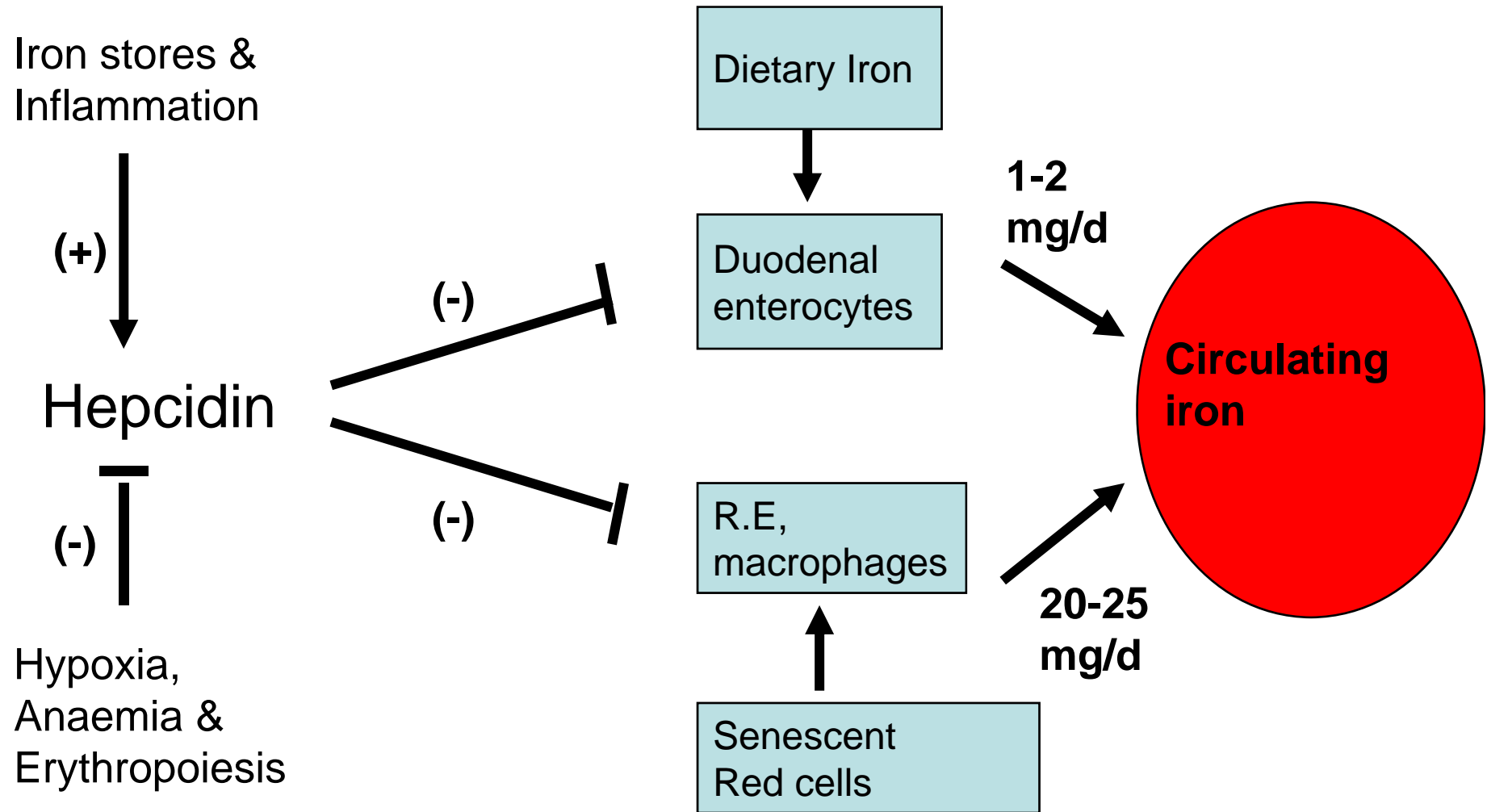


Darboe et al Lancet 2007;368:2088

The study of Darboe et al (2007) tells us:

- The level of morbidity did not change over the first 12 mo of life
- That is exposure to infection was constant
- The study was over two years that is seasonality did not influence the morbidity
- However the prevalence of chronic, sub-clinical inflammation (i.e. % raised AGP) increased with age
- That is infants were not fully recovered before the next bout of illness struck.

Control of iron homeostasis by hepcidin

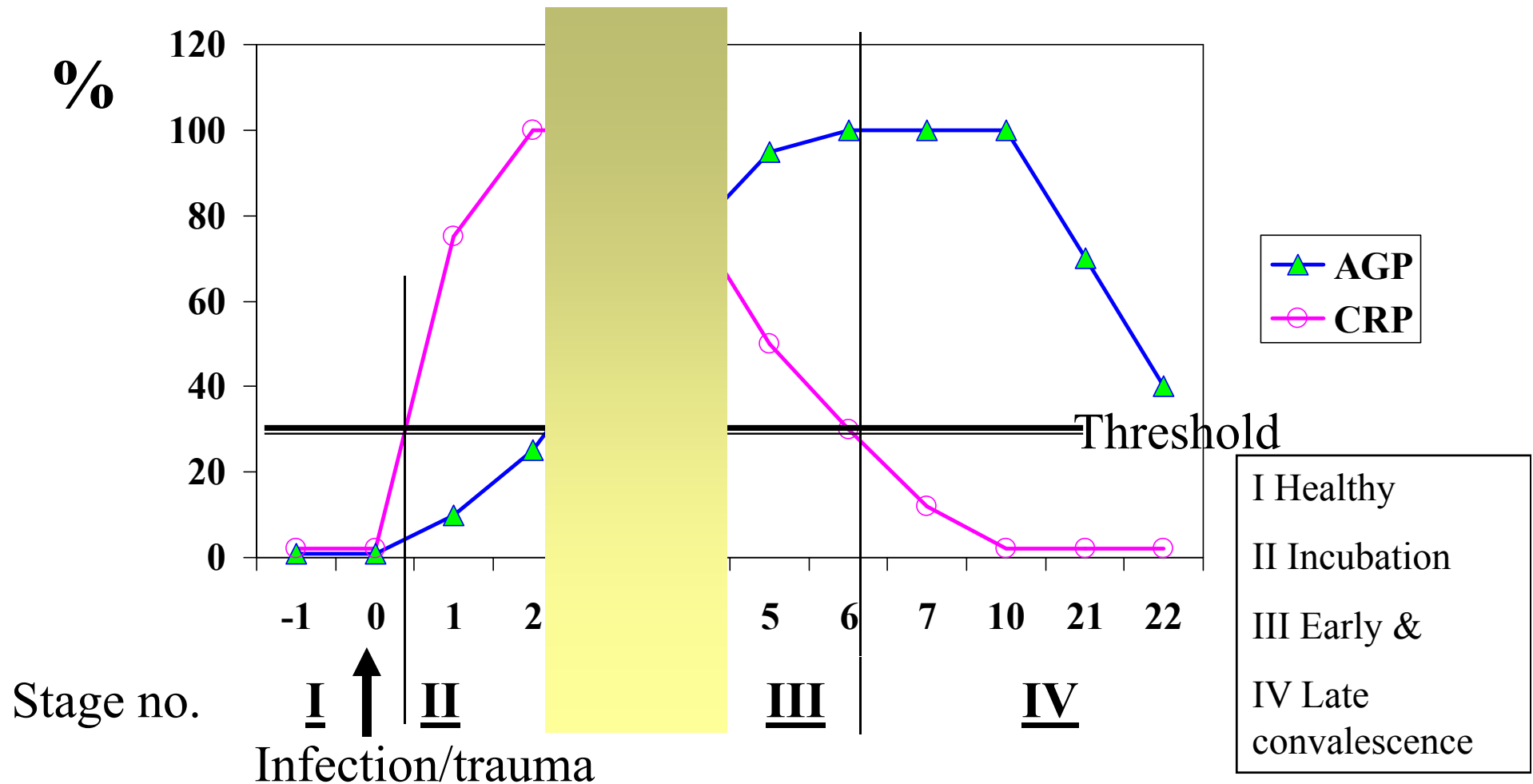


What can you do about the inflammation?

Minimise the influence of inflammation

- Discard samples where inflammation is detected
- Usual to measure C-reactive protein
- CRP is an acute phase protein and is a good indicator of acute inflammation
- It is not an indicator of chronic inflammation
- Exclusion potentially biases results
- We have developed a method to correct for inflammation

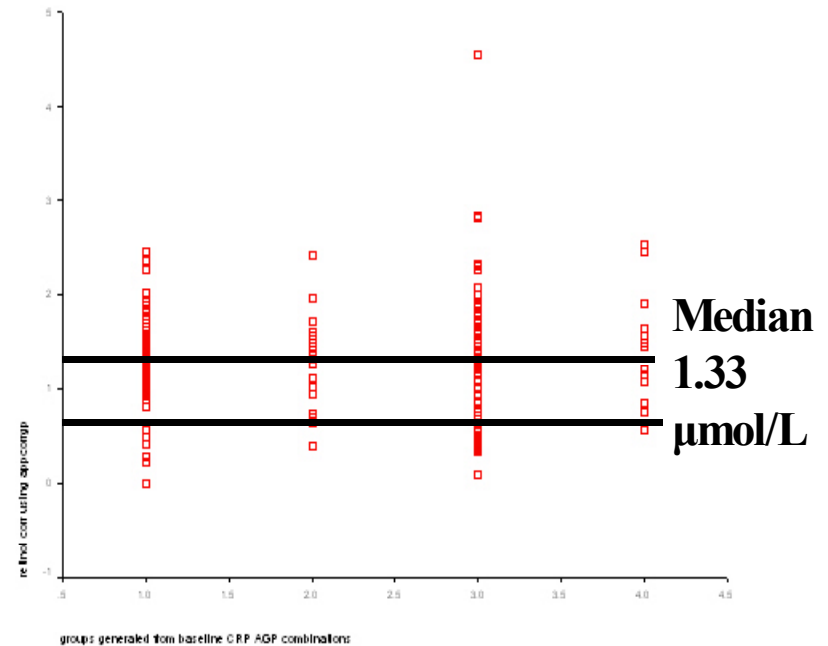
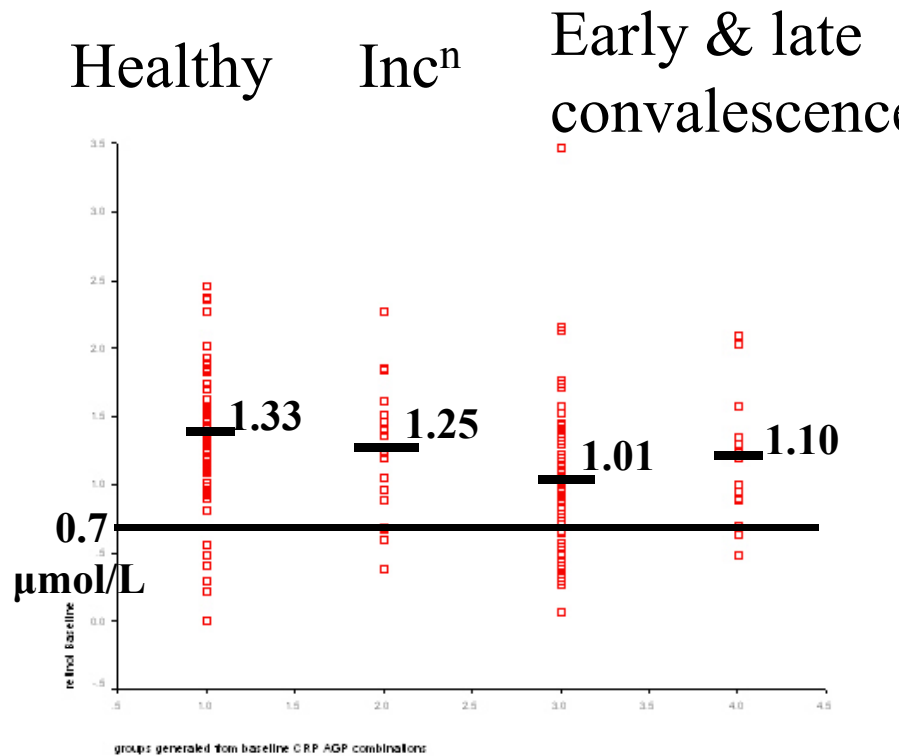
Time course of acute phase proteins following onset of infection



Principle of using 2 APP to correct nutrient biomarkers e.g. retinol

- Calculate medians of the four groups
- Let Reference group median = 15 &
- Let Incubation group median = 10
- Calculated ratio Incubation:Reference
- Equals 15:10 or 1.50
- That is on average nutrient concentration in incubation group is 50% less than those in reference group
- Multiply all nutrient concentrations in incubation group by 1.50

Influence of APP correction on plasma retinol concentrations



Corr. Factors 1.06 1.32 1.21

Calculating correction factors

- Unreliable if based on a single study
- Combined studies on apparently-healthy people with data on nutrient, AGP and CRP concentrations
- Meta-analysis for retinol, 15 studies, 13 infants and 2 women (Lancet 2003)
- Meta-analysis for ferritin, 22 studies, 5 infants, 3 children, 3 men and 11 women (Micronutrient Forum meeting Beijing 2009)

Calculating correction factors in meta-analysis

- Same principal as for individual study above except
- Used natural logs to calculate means of groups
- Weighted means for the different sample sizes
- Calculated the ratios

Correction factors from the meta-analyses

	Retinol	Ferritin
Incubation	1.15	0.763
Early convalescence	1.31	0.543
Late convalescence	1.12	0.741

Usefulness of correction factors

- You do not have to discard results
- Using correction factors provides you with a better estimate of the prevalence of nutrient deficiency
- Correction factors improve your ability to detect change in intervention studies

Intervention studies

- You cannot assume that level of inflammation will be the same at baseline and the end point.
- Environmental factors influence risk of infection and these vary from year to year.
- Correction factors enable you to remove the influence of inflammation from data and increase ability to detect change

Conclusions

- Several important nutrient biomarkers are altered by inflammation
- You cannot avoid inflammation
- It occurs in all communities
- Inflammation is greater where the frequency of infection is high
- Effects of inflammation on nutritional status are greatest in poor communities where quality of food is poor
- That is, it is more difficult to recover nutritional status following illness

Conclusion (2)

- Discarding data where inflammation is not an option in some communities
- Using CRP and AGP to identify inflammation has also enabled correction factors (CF) to be determined for retinol and ferritin
- CF allow better estimates of prevalence
- CF are particularly useful for intervention studies